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EasySC DNA Methylation Kit

Catalog Number: D200-1, D200-2

Table 1. Kit Components and Storage

Kit Component	D200-1 (50 preps)	D200-2 (200 preps)	Storage	Stability
CT Conversion Reagent*	5 Tubes	20 Tubes	Room Temperature	The product is stable for one year when stored as directed.
Dilution Buffer	1.5 mL	7 mL		
Dissolving Buffer	500 µL	1.2 mL		
Binding Buffer	30 mL	120 mL		
Wash Buffer**	6 mL	25 mL		
Desulphonation Buffer	10 mL	40 mL		
Elution Buffer	1.2 mL	5 mL		
Mini Column	50	200		
2 mL Collection Tube	50	200		

***** 900 µL water, 300 µL Dilution Buffer, and 50 µL Dissolving Buffer must be added per tube of **CT Conversion Reagent** prior to use.

****** Add 24 mL of 100% ethanol to the 6 mL **Wash Buffer** concentrate (D200-1) or 100 mL of 100% ethanol to the 25 mL **Wash Buffer** concentrate (D200-2) before use.

Product Description

EasySC DNA Methylation Kit integrates DNA denaturation and bisulfite conversion processes into one-step. Also, the kit has been streamlined for high yield recovery of DNA following DNA bisulfite conversion. EasySC DNA Methylation Kit provides innovative in-column desulphonation technology that eliminates cumbersome DNA precipitation steps while providing researchers consistent results every time. The kit is optimized to minimize template degradation, loss of DNA during treatment and clean-up, and to provide complete conversion of unmethylated cytosines. Recovered DNA is ideal for PCR amplification for downstream analyses including endonuclease digestion, sequencing, microarrays, etc.

Features

- ❖ Complete bisulfite conversion of DNA in less than 2 hours.
- ❖ Desulphonation and recovery of bisulfite-treated DNA with a spin column.
- ❖ Recovered DNA is ideal for downstream analyses such as PCR, endonuclease digestion, sequencing, microarrays, etc.

Reagent Preparation

▪ Preparation of CT Conversion Reagent

The **CT Conversion Reagent** supplied within this kit is a solid mixture and must be prepared prior to first use. Prepare as follows:

1. Add 900 µL water, 300 µL of **Dilution Buffer**, and 50 µL **Dissolving Buffer** to a tube of **CT Conversion Reagent**.

2. Mix at room temperature with frequent vortexing or shaking for 10 minutes.

   Note: It is normal to see trace amounts of undissolved reagent in the **CT Conversion Reagent**. Each tube of **CT Conversion Reagent** is designed for 10 separate DNA treatments.

   Storage: The **CT Conversion Reagent** is light sensitive, so minimize its exposure to light. For best results, the **CT Conversion Reagent** should be used immediately following preparation. If not used immediately, the **CT Conversion Reagent** solution can be stored overnight at room temperature, one week at 4°C, or up to one month at -20°C. Stored **CT Conversion Reagent** solution must be warmed to 37°C, then vortexed prior to use.

- **Preparation of Wash Buffer**

  Add 24 mL of 100% ethanol to the 6 mL **Wash Buffer** concentrate (D200-1) or 96 mL of 100% ethanol to the 24 mL **Wash Buffer** concentrate (D200-2) before use.

General Protocol

1. Add 130 µL of the **CT Conversion Reagent** to 20 µL of your DNA sample in a PCR tube. If the volume of the DNA sample is less than 20 µL, make up the difference with water. Mix the sample by flicking the tube or pipetting the sample up and down, then centrifuge the liquid to the bottom of the tube.
2. Place the sample tube in a thermal cycler and perform the following steps:

   Step1. 95 ^{o}C for 5 min
   Step2. 54 ^{o}C for 30 min
   Step3. 95 ^{o}C for 1 min
   Step4. 54 ^{o}C for 30 min
   Step5. 95 ^{o}C for 1 min
   Step6. 54 ^{o}C for 30 min
   Step7. 4 ^{o}C storage for up to 20 hours
   Note: The 4°C storage step is *optional*.
3. Add 600 µL of **Binding Buffer** to a **DNA Mini Column** and place the column into a provided **Collection Tube**.
4. Load the sample (from Step 2) into the **DNA Mini Column** containing the **Binding Buffer**. Close the cap and mix by inverting the column several times.
5. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through.
6. Add 100 µL of **Wash Buffer** to the column. Centrifuge at 10,000 x g for 30 seconds.
7. Add 200 µL of **Desulphonation Buffer** to the column and let stand at room temperature (20-30°C) for 15-20 minutes. After the incubation, centrifuge at 10,000 x g for 30 seconds.
8. Add 200 µL of **Wash Buffer** to the column. Centrifuge at 10,000 x g for 30 seconds. Add another 200 µL of **Wash Buffer** and centrifuge for an additional 30 seconds.
9. Empty the filtrate, and place the column back into the collection tube. Centrifuge at 12,000 x g for 2 min to remove the remaining EtOH.
10. Place the column into a new 1.5 mL microcentrifuge tube. Add 20 µL of **Elution Buffer** directly to the column matrix. Centrifuge for 30 seconds at 10,000 x g to elute the DNA.
11. Discard the column and store the DNA at -20ºC.

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